TITLE: Abnormalities in Human Brain Creatine Metabolism in Gulf War Illness Probed with MRS

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14. ABSTRACT
Abnormal levels of total creatine (tCr) in veterans with Gulf War Illness have been observed in prior studies. The goal of this research is to estimate amounts and ^1H transverse relaxation times (T2s) of the methyl peaks of the molecules phosphocreatine (PCr) and free creatine (Cr) in brains of ill and well control) Gulf War veterans using phosphorus (^{31}P) and proton (^1H) magnetic resonance spectroscopy (MRS). This will add more detailed and specific information about the previous preliminary reports of abnormal levels of these metabolites in brains of ill Gulf War veterans, validating this potential diagnostic marker and providing better understanding of underlying pathophysiology. Secondary goals are to measure amounts of adenosine triphosphate (ATP), inorganic phosphate (P_i), and magnesium ion (Mg^{2+}) and to estimate intracellular pH from ^{31}P MRS data. Year 1 progress and achievements included testing of a new dual-tuned ^{31}P-^1H head coil for 3T MRS, developing and optimizing protocols and parameters for ^{31}P and ^1H MRS, performing theoretical calculations using parameter estimates from the literature to characterize the creatine phosphokinase (CPK)-catalyzed chemical exchange of phosphate between PCr and Cr, and doing normal volunteer experiments to explore and determine the best 3T parameters for measuring bi-exponential ^1H T_2 relaxation of the methyl peak of tCr at 3.0 ppm.

15. SUBJECT TERMS
Gulf War Illness, brain creatine and phosphocreatine metabolism, ^1H and ^{31}P magnetic resonance spectroscopy (MRS), bi-exponential transverse (T_2) relaxation

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INTRODUCTION

The purpose of this research was to investigate creatine metabolism in brains of Gulf War Illness veterans classified into four groups (healthy controls and Haley Syndromes 1, 2, and 3) by estimating amounts of phosphocreatine (PCr) and free creatine (Cr) using ^{31}P (for basal ganglia and white matter centrum semiovale) and ^{1}H (for basal ganglia) magnetic resonance spectroscopy (MRS). Secondary goals were to measure ^{1}H T_2 relaxation times of the methyl resonances of PCr and Cr and to measure amounts of adenosine triphosphate (ATP), inorganic phosphate (P_i), and magnesium ion (Mg^{2+}) and to estimate intracellular pH from ^{31}P MRS data.

KEYWORDS

Gulf War Illness, brain creatine and phosphocreatine metabolism, ^{1}H and ^{31}P magnetic resonance spectroscopy (MRS), bi-exponential transverse (T_2) relaxation

OVERALL PROJECT SUMMARY

Task 1 was completely successfully ahead of schedule in Q1, and Task 2 was completed successfully on schedule in Q2. Tasks for the project are about 7 months behind schedule because of the difficulties encountered in getting the ^{31}P MRS experiments to work well on the 3T Philips scanner with multinuclear capability at the Advanced Imaging Research Center (AIRC), the limited time available on that MR system, and (recently) delays caused by the P.I.'s impending move to Georgia State University in Atlanta on 11/01/13. A total of 33 normal volunteer candidates are now on the recruitment roster for the preparatory pilot study (Task 3d). An extensive series of tests was done to get the ^{31}P coil of the dual-tuned ^{31}P-^{1}H head coil to perform reliably; ^{31}P protocol sequences and parameters were developed, tested, and finalized. Sequences and parameters for the ^{1}H protocol were also developed, tested, and finalized. This included experiments to define the 3T parameters for which the difficult task of measuring bi-exponential T_2 relaxation of the methyl peak of tCr at 3.0 ppm are optimized.

Task 1. Revision of IRB and consent form to include USAMRMC ORP-specific language, including local IRB and DoD review and approval (**months 1-5**)
> ➤ This task was completed successfully, ahead of schedule, upon initial award to UT Southwestern Medical Center. When the grant was transferred to Georgia State University when the P.I. moved there in November of 2013, local IRB approval was obtained in May of 2014 and USAMRMC ORP approval was obtained in mid-August of 2014.

Task 2. Ordering, design, construction, and delivery of Advanced Imaging Research (AIR) dual-tuned ^{31}P-^{1}H head coil (**months 1-5**)
> ➤ The dual-tuned ^{31}P-^{1}H head coil was ordered from AIR on 12/18/2012 and delivered on 02/06/2013; the annular loading phantom and holder for testing ^{31}P coil performance was delivered on 03/05/2013. Although the ^{1}H coil performance met specifications, no signal was detected with the ^{31}P coil upon initial testing, so the coil was returned to the vendor for repair. The quadrature combiner connections for ^{31}P were reversed and the modified coil was delivered on 03/15/2013. The modified ^{31}P coil was tested and again no signal was detected in either polarity/orientation in the magnet. Philips engineers, consulting with Cleveland headquarters where the coil

was originally tested, tested the UT Southwestern AIRC 3T Philips MR system multi-nuclear transmit and receive channels several times over the next 10 days, finally locating and correcting a cable connection problem. After again swapping the ^{31}P quadrature combiner connection to return them to their original configuration, the coil was again tested in the 3T magnet on 03/28/13 and this time the expected 31P signal was detected. However, intermittent problems continued over the following 3.5 months, so the coil was again returned to the vendor for repair on 07/19/13. An intermittent connection on a semi-rigid cable in the transmit-receive (TR) switching circuit of the ^{31}P receive chain was found and fixed. After the coil was shipped back on 08/06/13, phantom and human volunteer tests over the next three weeks indicated the intermittent problems were gone. These technical problems delayed the project by about six months.

Task 3. Development/testing of 3T Philips protocols, sequences, and parameters (**months 1-6**)
3a. Plan and set up MR scan protocols and sequences and optimize parameters (**months 1-5**).
  ➢ Both ^{1}H and ^{31}P protocols, sequences, and parameters were set up, tested, modified and optimized, and finalized, including extensive testing of the dual-tuned coil, with phantoms and with normal human volunteers (see 3b-3d below).
3b. Make phantoms for both ^{1}H and ^{31}P MRS sequence testing and testing of the AIR dual-tuned ^{31}P-^{1}H head coil (**months 1-5**).
 ➢ To augment available Philips and AIR phantoms, a 250-mL phantom initially containing 30 mM PCr, 15 mM ATP, and 16.5 mM $MgCl_2$ in 2% BSA to realistically mimic brain metabolite spectra was made for calibrating and testing the ^{31}P-^{1}H head coil and Philips 14-cm diameter ^{31}P surface coil with ISIS spatial localization.
  ➢ To test ability to measure bi-exponential relaxation of the tCr peak with ^{1}H MRS in order to resolve Cr and PCr, four phantoms were made. Each consisted of 20 mL of 2% bovine serum albumin (BSA) aqueous solution buffered with 50 mM HEPES and (1) 50 mM Cr, (2) 50 mM PCr, (3) 50 mM Cr and 50 mM PCr, (4) 50 mM Cr, 60 mM PCr, 30 mM ATP, 33 mM $MgCl_2$, 3500 units or 0.42 mg/mL of creatine phosphokinase (CPK), ph = 7.4. The 20 mL vials were placed in a 125 mL bottle of 150 mM saline to reduce susceptibility artifacts near the vial walls and permit better shimming to narrower line widths and thus suppression of the water signal
3c. Collect phantom data to characterize and verify performance of the dual-tuned ^{31}P-^{1}H head coil, run final tests of protocols and sequences, do final parameter optimizations (**month 6**).
 ➢ Calibration and sensitivity tests were performed on the ^{1}H and ^{31}P circuits of the dual-tuned AIR ^{31}P-^{1}H head coil and compared with the Philips P140 14-cm ^{31}P surface coil, using both Philips-AIR and homebuilt phantoms.
  ➢ Although the ^{1}H sensitivity was only about half that specified by the manufacturer of the coil, the ^{1}H channel sensitivity was adequate for imaging to position voxels, for shimming to provide narrow line widths, and for decoupling to collapse ^{31}P-^{1}H spin-spin coupling in ^{31}P spectra. The 8-channel and 32-channel Philips ^{1}H head coils had better ^{1}H sensitivity, and were used for ^{1}H MRS data collection.
  ➢ The ^{31}P performance of the dual-tuned AIR-SREE coil typically met the limited manufacturer specifications, but intermittent sensitivity issues led to several rounds of trouble-shooting and repairs of both the coil and the MR scanner in a six-month period before performance became reliable.
  ➢ After these adjustments and modifications were made, both ^{1}H and ^{31}P protocols and parameters were finalized.
3d. Test the entire MRS protocol on 4 normal volunteers as a preparatory pilot study (**month 6**).

- A total of 24 human protocols tests were performed using 8 subjects at UT Southwestern Medical Center, 12 with ^{1}H (one at 7T) and 12 with ^{31}P, to optimize the protocols and parameters. This phase of the project was completed successfully, albeit 5 months behind schedule, for both ^{31}P (see following bullet point and Sub-section A of "Problems" section below) and ^{1}H (see second bullet point below).
- *Final ^{31}P protocol and sequence parameters*: Even after fixing scanner and coil problems causing intermittent sensitivity deficiency, ^{31}P gave lower SNR performance than had been anticipated, necessitating use of more signal averages, a TR (2-3 s) shorter than the 20 s needed for complete T_1 relaxation, and a voxel volume of at least 100 mL for adequate SNR in a reasonable time. This precluded including basal ganglia as originally proposed. A bilateral white matter centrum semiovale voxel gave acceptable though not good 11-minute ^{31}P spectra under these conditions (see Figure 1 below).
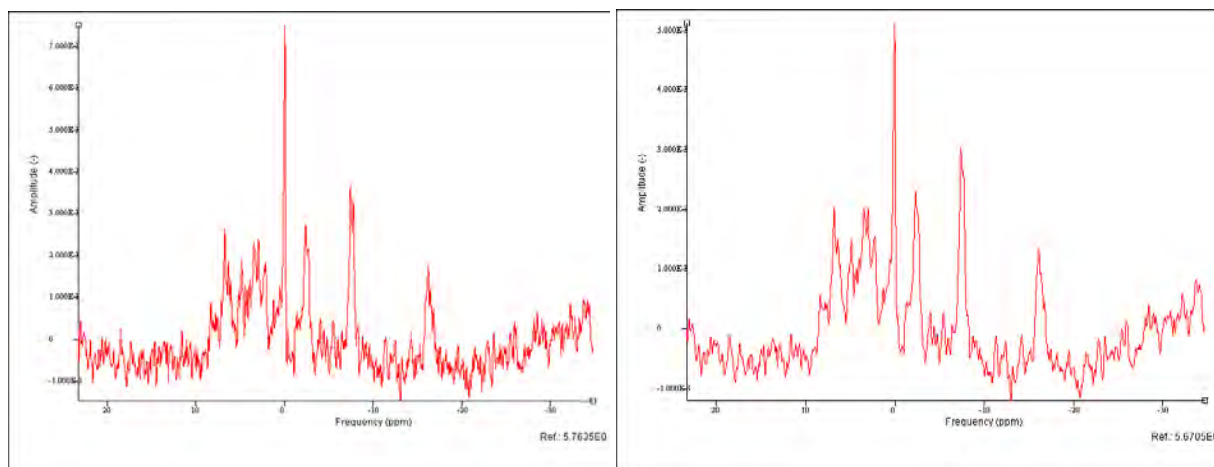
4_1, TR = 2 s, NSA = 320, duration = 10:48, rms B_1 = 2.35 μT
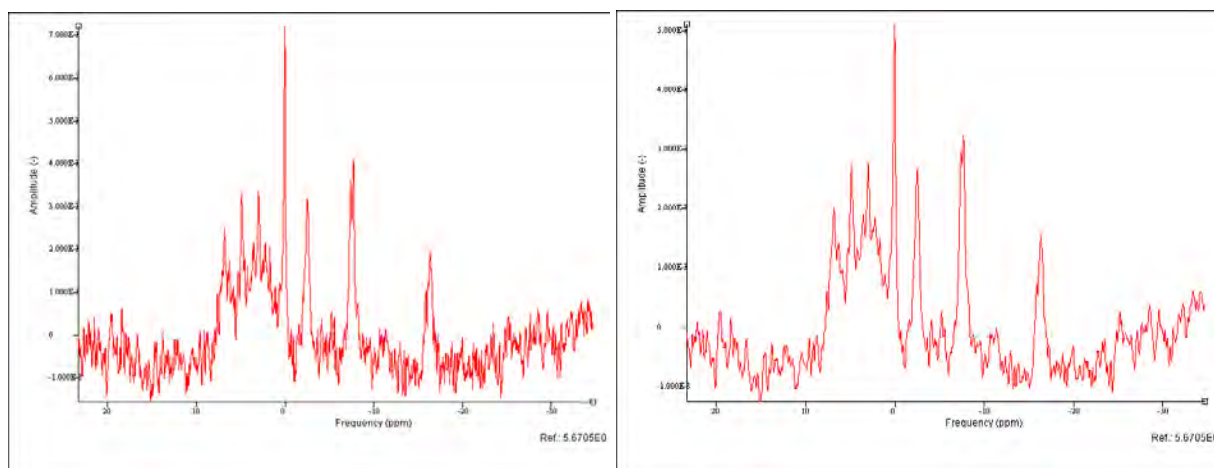
5_1, TR = 3s, NSA = 216, duration = 10:57, rms B_1 = 1.92 μT
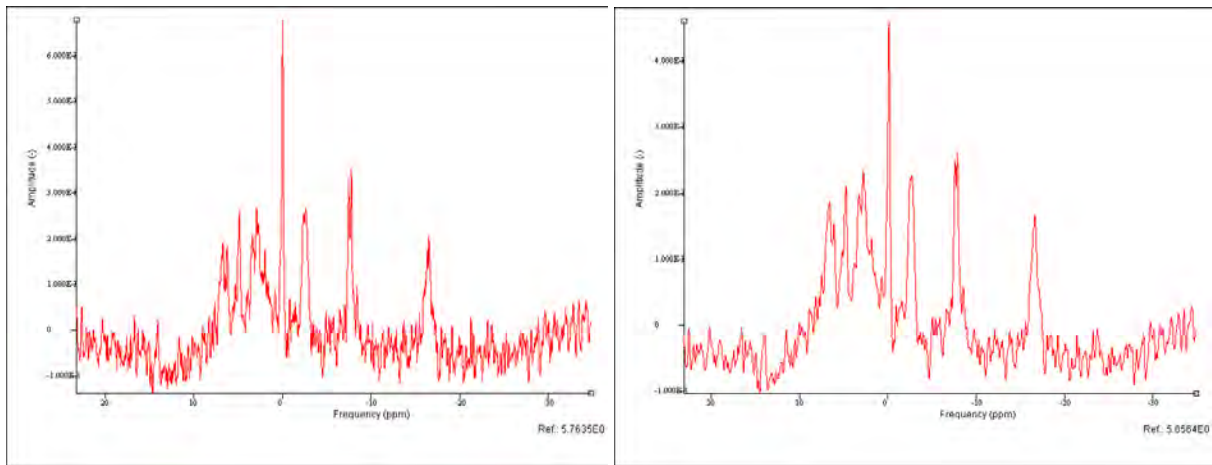
6_1, TR = 2 s, NSA = 320, duration = 10:48, rms B_1 = 2.35 µT

Figure 1. 08/09/13 3T6594 ^{31}P ISIS MRS spectra using AIR/SREE dual-tuned head coil (after repair). Bilateral (mostly) white matter (WM) centrum semiovale, 5.5 (A-P) x 7.0 (R-L) x 2.5 (I-S) = 96.3 cm^3, 4 disdacqs, TE = min (0.20 ms), offset frequency = -250 Hz, SW = 3000 Hz, 2048 data points zero-filled to 4096, RO duration = 682.7 ms, broadband WALTZ decoupling (max B1 = 3 µT, offset frequency = -100 Hz), LB = 15 Hz (left) and 30 Hz (right). Tallest peak is PCr, three right-most peaks are ATP.
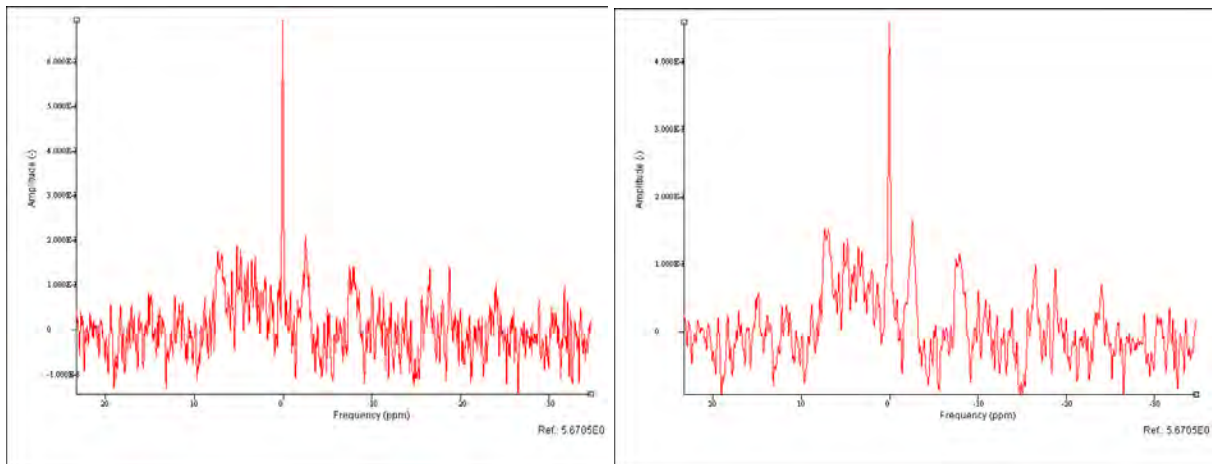
Bilateral cerebellum (4_1) voxel, 3.5 (A-P) x 8.0 (R-L) x 3.0 (I-S) = 84.0 cm^3, 2 oblique coronal 15-mm REST slabs.
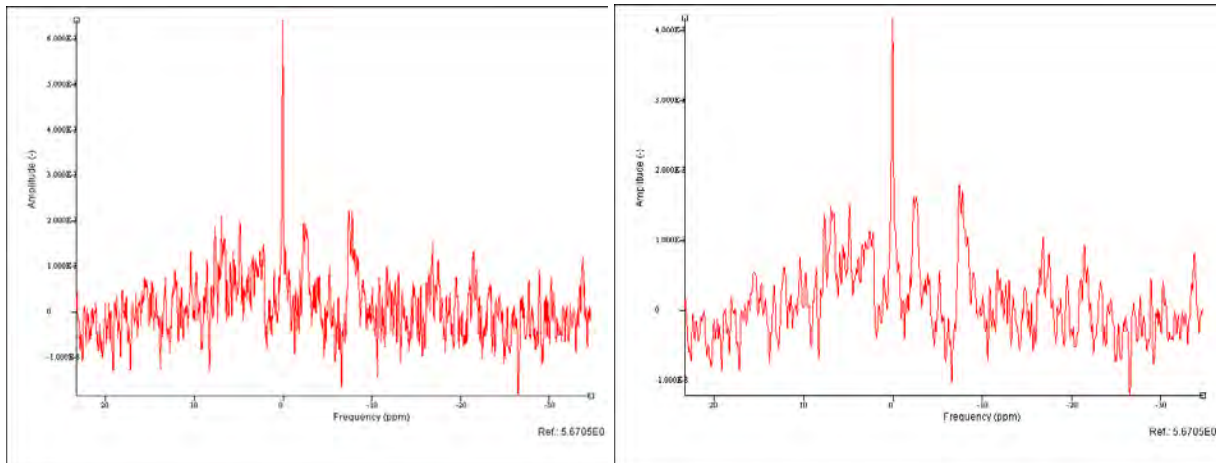
Bilateral frontal_ACC (5_1) voxel, 4.0 (A-P) x 6.0 (R-L) x 3.0 (I-S) = 72.0 cm^3, 2 oblique axial 15-mm REST slabs.

7
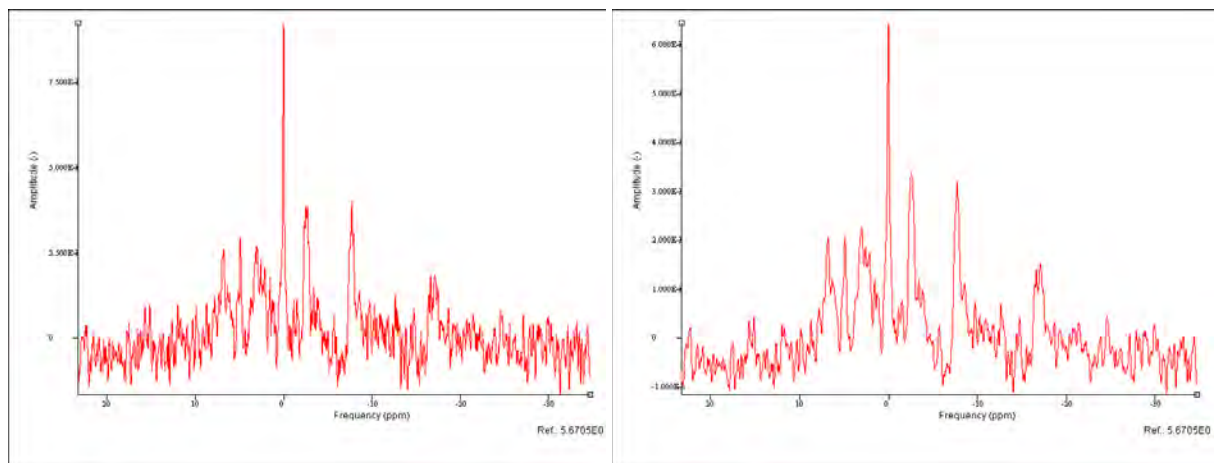
Bilateral (mostly) WM centrum semiovale (6_1) voxel, 5.5 (A-P) x 7.0 (R-L) x 2.5 (I-S) = 96.3 cm^3

**Figure 2.** 08/07/13 3T6586 ^{31}P ISIS MRS using AIR/SREE dual-tuned head coil (after repair). TR = 4 s and NSA = 144, duration = 9:44, TE = min (0.10 ms), 2 disdacqs, SW = 3000 Hz, 2048 data points zero-filled to 4096, LB = 15 Hz (left) and 30 Hz (right). Tallest peak is PCr, three right-most peaks are ATP.

But 10-minute ^{31}P spectra obtained from slightly smaller voxels of bilateral frontal lobe including anterior cingulate cortex (ACC) and of bilateral cerebellum (see Figure 2) were of lower quality. The rationale for frontal lobe was that Lac and Glx change there with exercise differs in two groups of ill Gulf War veterans (GWV) with chronic fatigue syndrome (CFS) that respond differently to exercise stress (1). The rationale for cerebellum was that only striatum and hippocampus have higher acetylcholinesterase (AChE) activity in rat brain, from an organophosphate (OP) toxicity study (2).

➢ *Final ^{1}H protocol and sequence parameters*: Sequences for ^{1}H MRS data were tested on human volunteers with the 8-channel and 32-channel Philips head coils on the two 3T Philips MR scanners at UT Southwestern's Advanced Imaging Research Center (AIRC). The two coil types gave similar signal-to-noise ratio (SNR). Using a water saturation bandwidth of 75 Hz, MOIST water suppression worked slightly better and more reliably than either VAPOR or CHESS. Voxel sizes and locations and number of spectral averages (NSA) needed to obtain spectra with adequate SNR within the 1.25 hours allotted for the ^{1}H session were tested and verified. The TR of 8 s provides fully relaxed spectra with no dependence of signal intensity upon T_1 for both metabolites and water. With TE = 30 ms, there is negligible effect of T_2 on metabolite quantification. Voxels selected were: **(1)** left basal ganglia, 12 mL, (30 mm A-P x 20 mm R-L x 20 mm I-S), NSA = 64, duration = 8:32; **(2)** left white matter centrum semiovale, 12 mL (45 mm A-P x 18 mm R-L x 15 mm I-S), NSA = 32, duration = 4:16; **(3)** left anterior cingulate, 6 mL (40 mm A-P x 13 mm R-L x 12 mm I-S), NSA = 64, duration = 8:32; **(4)** left cerebellum, nominally 22.5 mL (25 mm A-P x 30 mm R-L x 30 mm I-S), NSA = 24, duration = 3:12. These times do not include placement of voxel positions, shim and water suppression optimization, and collection of non-suppressed water reference spectra, which bring the experiment duration to slightly more than an hour. Two cerebellum voxels were initially tested, one mainly white matter and one mainly gray matter, but difficulties in reliably shimming to a narrow Lorentzian line and effectively suppressing water signal in the multiply oblique voxels, as well as time constraints, led to the conclusion that a single cerebellum voxel would be more practical. Representative ^{1}H spectra from these four

brain regions are shown in Figure 3 below. Good water suppression (see suppressed water peak intensities at 4.6 ppm in left panels of Figure 3) and metabolite SNR were routinely obtained.
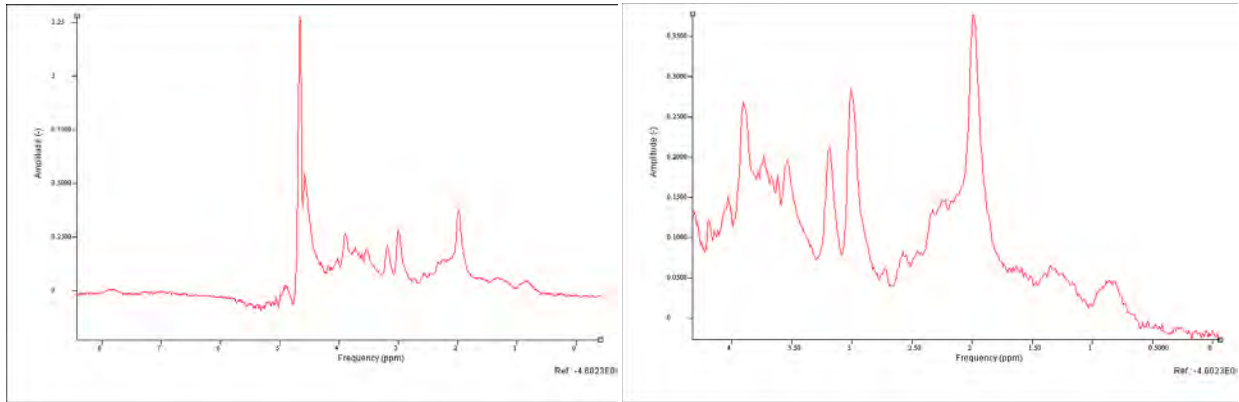
Left basal ganglia (BG), 3.0 (A-P) x 2.0 (R-L) x 2.0 (I-S) = 12.0 cm^3, 32-channel head coil, 3TB1527 normal control (NC) volunteer (07/12/13), NSA = 64, t_{acq} = 8:32
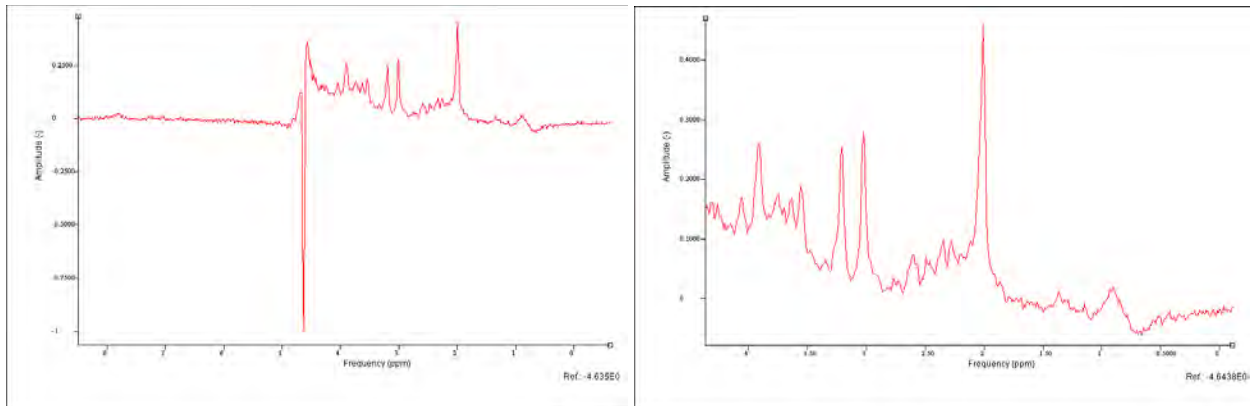
Left white matter (WM) centrum semiovale, 4.5 (A-P) x 1.72 (R-L) x 1.5 (I-S) = 11.61 cm^3, 34° A-P/10° R-L/-9° I-S oblique, 32-channel head coil, 3TB1527 normal control (NC) volunteer (07/12/13), NSA = 32, t_{acq} = 4:16
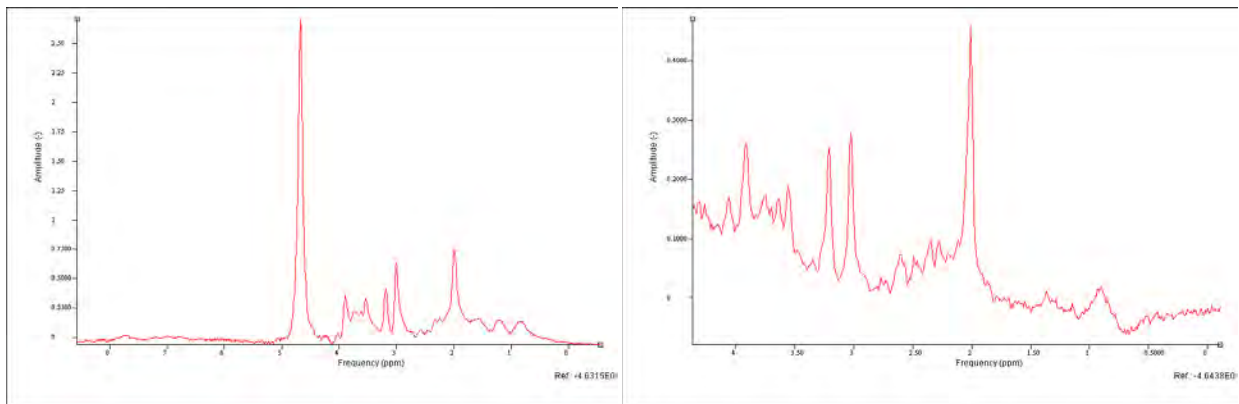
Left cerebellum, 2.5 (A-P) x 3.0 (R-L) x 3.0 (I-S) = 22.5 cm^3, 16° A-P oblique, 32-channel head coil, 3TB1527 normal control (NC) volunteer (07/12/13), NSA = 24, t_{acq} = 3:12
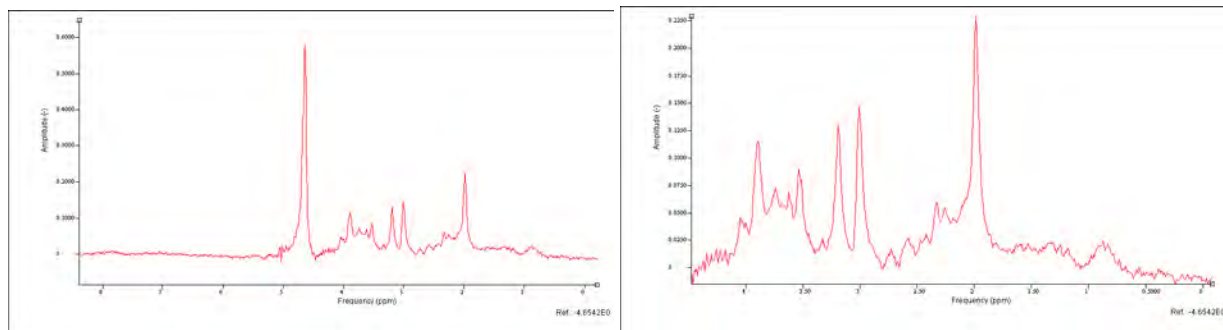
Left anterior cingulate cortex (ACC), 4.0 (A-P) x 1.5 (R-L) x 1.2 (I-S) = 7.2 cm^3, 2° A-P/25° R-L/4° I-S oblique, 8-channel head coil, 3T6463 normal control (NC) volunteer (06/20/13), NSA = 64, t_{acq} = 8:32

Figure 3. Typical 3T 1H MRS from four brain regions obtained with TR = 8 s, TE = 31-32 ms, SW = 2 kHz, 1024 →2048points, 75 Hz MOIST water suppression, LB = 3Hz. Left panels show water suppression quality, right panels are expanded to show only metabolite signals. Methyl peaks of NAA (2.0 ppm), Cr (3.0 ppm), and Cho (3.2 ppm) are most prominent.

The rationale for including WM centrum semiovale is that white matter damage has been reported in Gulf War Illness (GWI) (3-6). The rationale for including ACC is that Lac and Glx changes with exercise differ in two groups of ill GWV with CFS that respond differently to exercise stress (1). The rationale for adding cerebellum is that only striatum and hippocampus have higher AChE activity in rat brain, from an organophosphate (OP) toxicity study (2).

➢ *Experimental results for bi-exponential T_2 for tCr (and other) peaks*: Multi-TE experiments were done with normal volunteers to determine if bi-exponential decay curves for the total creatine (tCr, or PCr + Cr) methyl peak could be observed, as reported in one paper in the literature (7). Most data were collected from a white matter voxel in left hemisphere centrum semiovale, to ensure that bi-exponential decay from tCr in gray matter and tCr in white matter was not mistaken for bi-exponential decay from PCr and Cr. Some data were collected from a parietal voxel containing nearly equal amounts of gray matter and white matter, to see if bi-exponential behavior of tCr due to tissue type could be detected (8). At 1.5T, T_2 has been reported to be longer in WM than in GM for NAA (483 ± 20 ms cp. 399 ± 9 ms), similar in WM and GM for tCr (209 ± 5 ms cp. 204 ± 2 ms), and shorter in WM than in GM for tCho (325 ± 10 ms cp. 401 ± 20 ms) (8). To check the effect of differing minimum TE values on the ability to detect and quantify the faster relaxing component, minimum TE was varied between 30 and 50 ms using a PRESS localization sequence and a few experiments were done with STEAM localization to lower the minimum TE still further, to 10 ms. The effect of minimum TE on fit results was also investigated by systematically ignoring initial (short) TE values in the fitting procedure. Longest TE values were selected to ensure that signal had decayed nearly completely, to improve the fit quality for the longer T_2 component. To check if inadequate signal-to-noise might lead to fitting of a very long T_2 component artifact from the long-TE tail of the decay curve, a noise floor or baseline asymptote parameter was included in some of the fits. Finally, since T_2 values were expected to become shorter at higher field strength, and our 3T data were being evaluated in light of prior literature work reporting distinguishably different PCr and Cr T_2 values at 1.5 T (7) but not at 4T (9), a multiple-TE experiment was also conducted at 7T.

Relaxation data in Tables 1-5 were collected at 3T with 32 signal averages and TR = 3.0 s and required about 75 minutes. Using jMRUI, time-domain FIDs were zero-filled and multiplied with an exponential function yielding 3 Hz Lorentzian broadening before Fourier transformation and phasing. Table 1 shows T_2 values calculated for the 3.0 ppm tCr` methyl peak region with a 1-exponent, 2-parameter fitting equation ($y = ae^{-bx}$) and a 2-exponent, 4-parameter fitting equation ($y = ae^{-bx} + ce^{-dx}$) and, in the rows shaded in gray, with 1-exponent, 3-parameter fitting equation ($y = y_0 + ae^{-bx}$) and a 2-exponent, 5-parameter fitting equation ($y = y_0 + ae^{-bx} + ce^{-dx}$), where y_0 is a baseline asymptote or noise floor parameter.

Table 1. 3T T_2 values of total creatine (tCr) methyl peak at 3.0 ppm from single-exponential fit [e] and a double-exponential fit [f] of peak intensity decay curves from normal human volunteer subjects.

Subject	Date	Voxel [d]	TE Values	1-exponent tCr T_2 /ms [a/y_0]	2-exponent tCr T_{2A} /ms (f_A)	tCr T_{2B} /ms (f_B)	(a+c)/ y_0
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm³	40 log-spaced, 50-700 ms	155.2 ± 2.5	147.0 ± 10.9 (98.3%)	1x10⁸ ± 4.5x10⁷% (1.7%)	
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm³	40 log-spaced, 50-700 ms	147.0 ± 4.7 [57]	147.0 ± 0.0002 (57.3%)	147.0 ± 0.0002 (42.7%)	57
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	44 log-spaced, 35-900 ms	146.9 ± 2.1	281.8 ± 228.4 (20.6%)	116.9 ± 28.0 (79.4%)	
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	44 log-spaced, 35-900 ms	135.2 ± 3.3 [e] [45]	142.5 ± 5.4 [f] (51.9%)	11.5 ± 8.3 [f] (48.1%)	102.5
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 32-700 ms	174.2 ± 4.0	174.2 ± 0.02 (58.1%)	174.2 ± 0.02 (41.9%)	
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 32-700 ms	177.0 ± 8.6 [170]	177.0 ± 6x10⁵% (53.9%)	177.0 ± 7x10⁵% (46.1%)	170
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	40 log-spaced, 32-700 ms	157.1 ± 4.1	130.75 ± 6.5 (94.4%)	9x10⁶ ± 3x10⁸% (5.6%)	
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	40 log-spaced, 32-700 ms	130.7 ± 6.1 [e] [17]	130.7 ± 0.03 [f] (56.25%)	130.7 ± 0.001 [f] (43.75%)	17
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	175.0 ± 6.0	244.1 ± 27.8 (56.3%)	23.6 ± 8.0 (43.7%)	
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	113.5 ± 13.8 [e] [8]	284 ± 120 [f] (55.9%)	24.6 ± 9.5 [f] (44.1%)	30
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	146.2 ± 4.6	272.8 ± 126.8 (38.35%)	85.8 ± 27.9 (61.65%)	
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	122.0 ± 6.1 [e] [36]	329 ± 1008 [f] (33.8%)	89.5 ± 56.9 [f] (66.2%)	69.5

[a] Subject uncomfortable, talking, and moving intermittently last 15 minutes of scan; poor quality in 25% of data.
[b] Subject uncomfortable last 10 minutes of scan, stopped with 3 minutes left for a restroom break, did not get data for last two TE values.
[c] PRESS used for localization on all except 07/26/13 and 08/16/13 Subject #1 scans, when STEAM was used.
[d] All voxels in left hemisphere.
[e] Used a 1-exponent, 2-parameter fit ($y = ae^{-bx}$) in odd rows and a 1-exponent, 3-parameter fit ($y = y_0 + ae^{-bx}$) in even, gray-shaded rows; signal-to-noise = a/y_0 in the latter.
[f] Used a 2-exponent, 4-parameter fit ($y = ae^{-bx} + ce^{-dx}$) in odd rows and a 2-exponent, 5-parameter fit ($y = y_0 + ae^{-bx} + ce^{-dx}$) in even, gray-shaded rows; signal-to-noise = $(a + c)/y_0$ in the latter.

In one case (Subject 2), the bi-exponential fit yielded components with the same T_2 value. In two cases (Subject 3 and 07/10/13 session of Subject 1), the second component either constituted only a small percentage of the total 3.0 ppm signal intensity and had an unrealistically long T_2 value (without the extra y_0 parameter) or had the same T_2 value as

the first component (with the extra y_0 parameter). In the remaining three cases, the second component was a substantial fraction (44-79%) of the total 3.0 ppm signal intensity, with shorter T_2 values of 12-117 ms, not dissimilar from Ke et al. 2002 (7). Two of these three cases were those in which the shortest TE was 10 ms, achieved with STEAM localization, 20 ms shorter than achievable with PRESS localization. Including a baseline asymptote or noise floor parameter in the one- and two-exponential fit equations significantly shortened the calculated T_2 of both components for the single PRESS experiment where bi-exponential behavior was observed, but had little effect (both component T_2 values slightly lengthened) for the two STEAM experiments with shorter minimum TE.

The effect of minimum TE in PRESS data was also investigated by systematically ignoring initial (short) TE values in the fitting procedure (Table 2). This produced longer single-exponential T_2 values. For bi-exponential fits, ignoring the shortest TE datum of 35 ms lengthened the calculated T_2 for both components, but omitting data for TE values of 40 ms and longer resulted in replacement of the shorter-T_2 second component with an unrealistically long T_2 component comprising only a few percent of the total 3.0 ppm signal intensity and a concomitant shortening of the first component T_2 to values near those obtained with a single-exponential equation fit. This is similar to the result obtained in the 07/10/13 session of Subject 1 (Table 1), with minimum TE of 50 ms. It indicates that TE values shorter than 40 ms are needed to observe bi-exponentiality here.

Table 2. 3T T_2 values of total creatine (tCr) methyl peak at 3.0 ppm from single- and double-exponential fits of peak intensity decay curves, ignoring initial data points, from PRESS of normal volunteer subject.

Subject	Date	Voxel [e]	TE Values	1-exp, 2-par tCr T_2 /ms	2-exp, 4-par tCr T_{2A} /ms (f_A)	2-exp, 4-par tCr T_{2B} /ms (f_B)
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	44 log-spaced, 35-900 ms	146.9 ± 2.1	281.8 ± 228.4 (20.6%)	116.9 ± 28.0 (79.4%)
1 [a] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	43 log-spaced, 40-900 ms	147.7 ± 2.2	373.4 ± 478.0 (11.8%)	124.7 ± 22.7 (88.2%)
1 [b] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	42 log-spaced, 44-900 ms	149.9 ± 2.1	139.4 ± 1.5 (97.9%)	$9.5 \times 10^9 \pm 5 \times 10^8$% (2.1%)
1 [c] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	41 log-spaced, 49-900 ms	151.5 ± 2.0	142.0 ± 4.0 (98.1%)	$9.9 \times 10^8 \pm 9 \times 10^7$% (1.9%)
1 [d] (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	40 log-spaced, 54-900 ms	152.6 ± 2.1	143.5 ± 3.8 (98.3%)	$8.8 \times 10^9 \pm 2.5 \times 10^8$% (1.7%)

[a] Ignoring shortest TE value (TE = 35 ms) data.
[b] Ignoring two shortest TE values (TE = 35 ms, 40 ms).
[c] Ignoring three shortest TE values (TE = 35 ms, 40 ms, 44 ms).
[d] Ignoring four shortest TE values (TE = 35 ms, 40 ms, 44 ms, 49 ms).
[e] Voxel in left hemisphere.

The effect of minimum TE in STEAM data was also investigated by systematically ignoring initial (short) TE values in the fitting procedure (Table 3). This produced little change in single-exponential T_2 values until TE values of 30-50 ms were excluded, whereupon calculated T_2 values progressively lengthened. For bi-exponential fits, ignoring the shortest TE data shortened the long-T_2 component, increasing its contribution as TEs of 10-20 ms were successively ignored and then decreasing it as TEs of 20-40 ms were ignored. When the minimum TE was greater than 50 ms, a single exponential result was approached, with a small residual component with an unrealistically long T_2.

Table 3. 3T T_2 values of total creatine (tCr) methyl peak at 3.0 ppm from single- and double-exponential fits of peak intensity decay curves, ignoring initial data points, from STEAM of normal volunteer subject.

Subject	Date	Voxel [i]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCr T_2 /ms	tCr T_{2A} /ms (f_A)	tCr T_{2B} /ms (f_B)
1 (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	146.2 ± 4.6	272.8 ± 126.8 (38.35%)	85.8 ± 27.9 (61.65%)
1 [a] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	39 log-spaced, 15-600 ms	145.7 ± 4.8	227.8 ± 55.6 (51.6%)	65.6 ± 22.9 (48.4%)
1 [b] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	38 log-spaced, 20-600 ms	146.7 ± 5.2	209.8 ± 35.45 (57.4%)	52.7 ± 19.45 (42.6%)
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	37 log-spaced, 25-600 ms	147.8 ± 5.5	193.2 ± 19.9 (59.6%)	36.2 ± 13.2 (40.4%)
1 [d] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	36 log-spaced, 30-600 ms	150.0 ± 5.9	184.3 ± 12.9 (51.6%)	23.9 ± 8.4 (48.4%)
1 [e] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	35 log-spaced, 36-600 ms	154.9 ± 6.1	182.3 ± 12.1 (44.6%)	20.2 ± 8.7 (55.4%)
1 [f] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	34 log-spaced, 42-600 ms	158.4 ± 6.5	180.5 ± 9.7 (18.7%)	13.3 ± 5.8 (81.3%)
1 [g] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	33 log-spaced, 47-600 ms	163.4 ± 6.8	183.3 ± 10.2 (15.2%)	13.3 ± 6.6 (84.8%)
1 [g] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	32 log-spaced, 53-600 ms	171.4 ± 6.3	144.05 ± 45.1 (93.2%)	1133 ± 8350 (6.8%)

[a] Ignoring shortest TE value (TE = 10 ms) data.
[b] Ignoring two shortest TE values (TE = 10 ms, 15 ms).
[c] Ignoring three shortest TE values (TE = 10 ms, 15 ms, 20 ms).
[d] Ignoring four shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms).
[e] Ignoring five shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms).
[f] Ignoring six shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 36 ms).
[g] Ignoring seven shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 36 ms, 42 ms).
[h] Ignoring eight shortest TE values (TE = 10 ms, 15 ms, 20 ms, 25 ms, 30 ms, 36 ms, 42 ms, 47 ms).
[i] Voxel in left hemisphere.

For comparison and to serve as controls, methyl peaks of tCho at 3.2 ppm (Table 4) and NAA at 2.0 ppm (Table 5) were analyzed in the same way as for tCr (Table 1).

For tCho (Table 4), in three cases (Subjects 2 and 3 and the 07/10/13 session of Subject 1), the bi-exponential fit yielded components with the same T_2 value. In the remaining three cases, the second component was a substantial fraction (31-92%) of the total 3.2 ppm signal intensity, with shorter T_2 values of 8-99 ms. Two of these three cases were those in which the shortest TE was 10 ms, achieved with STEAM localization, 20 ms shorter than achievable with PRESS localization.

For NAA (Table 5), in two cases (Subject 2 and the 07/10/13 session of Subject 1), the bi-exponential fit yielded components with the same T_2 value. Since the primary co-resonant 2.0 peak is a small contribution from NAAG, this was expected. In the remaining four cases, the second component was a substantial fraction (38-84%) of the total 3.2 ppm signal intensity, with shorter T_2 values of 5-17 ms. Two of these four cases were those in which the shortest TE was 10 ms, achieved with STEAM localization, 20 ms shorter than achievable with PRESS localization.

Table 4. 3T T_2 values of total choline (tCho) methyl peak at 3.2 ppm from single- and double-exponential fits of peak intensity decay curves from normal human volunteer subjects.

Subject	Date	Voxel [d]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCho T_2 /ms	tCho T_{2A} /ms (f_A)	tCho T_{2B} /ms (f_B)
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm³	40 log-spaced, 50-700 ms	206.05 ± 3.55	206.06 ± 2.4x10⁶% (54.5%)	206.06 ± 2.4x10⁶% (45.5%)
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	44 log-spaced, 35-900 ms	222.9 ± 3.3	234.2 ± 6.5 (7.7%)	7.6 ± 3.5 (92.3%)
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 32-700 ms	221.1 ± 7.5	221.1 ± 6.5x10⁵% (63.2%)	221.1 ± 6.5x10⁵% (36.8%)
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	40 log-spaced, 32-700 ms	247.8 ± 8.8	247.8 ± 9.5x10⁵% (78.9%)	247.8 ± 9.5x10⁵% (21.1%)
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	217.5 ± 15.0	266.6 ± 35.7 (68.8%)	24.4 ± 16.0 (31.2%)
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	168,5 ± 5.8	350.5 ± 211.5 (35.7%)	98.6 ± 33.4 (64.3%)

[a] Subject uncomfortable, talking, and moving intermittently last 15 minutes of scan; poor quality in 25% of data.
[b] Subject uncomfortable last 10 minutes of scan, stopped with 3 minutes left for a restroom break, did not get data
 for last two TE values.
[c] PRESS used for localization on all except 07/26/13 and 08/16/13 Subject #1 scans, when STEAM was used.
[d] All voxels in left hemisphere.

Table 5. 3T T_2 values of N-acetylaspartate (NAA) methyl peak at 2.0 ppm from single- and double-exponential fits of peak intensity decay curves from normal human volunteer subjects.

Subject	Date	Voxel [d]	TE Values	1-exp, 2-par	2-exp, 4-par	
				tCr T_2 /ms	NAA T_{2A} /ms (f_A)	NAA T_{2B} /ms (f_B)
1 (M, 62y)	07/10/13	WM centrum semiovale 4.0x2.0x2.0 = 16.0 cm³	40 log-spaced, 50-700 ms	285.0 ± 0.9	285.0 ± 1x10⁷% (51.4%)	285.0 ± 1x10⁷% (48.6%)
1 (M, 62y)	07/15/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	44 log-spaced, 35-900 ms	214.3 ± 6.2	238.8 ± 3.9 (15.95%)	10.9 ± 4.9 (84.05%)
2 [a] (F, 41y)	07/19/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 32-700 ms	268.0 ± 9.3	268.0 ± 1.6x10⁷% (54.1%)	268.0 ± 1.6x10⁷% (45.9%)
3 [b] (F, 51y)	07/19/13	WM/GM parietal 4.0x2.0x3.0 = 24.0 cm³	40 log-spaced, 32-700 ms	184.1 ± 8.6	227.95 ± 7.25 (21.7%)	13.8 ± 2.0 (78.3%)
1 [c] (M, 62y)	07/26/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	364.3 ± 38.1	425.7 ± 40.4 (17.3%)	4.7 ± 1.8 (82.7%)
1 [c] (M, 62y)	08/16/13	WM centrum semiovale 4.0x1.8x1.8 = 13.0 cm³	40 log-spaced, 10-600 ms	214.2 ± 8.7	262.8 ± 6.1 (61.9%)	17.1 ± 1.9 (38.1%)

[a] Subject uncomfortable, talking, and moving intermittently last 15 minutes of scan; poor quality in 25% of data.
[b] Subject uncomfortable last 10 minutes of scan, stopped with 3 minutes left for a restroom break, did not get data
 for last two TE values.
[c] PRESS used for localization on all except 07/26/13 and 08/16/13 Subject #1 scans, when STEAM was used.
[d] All voxels in left hemisphere.

Optimal data conditions for successful and reliable fitting of bi-exponential data have been explored and defined (10-12) and determined to be a signal contribution ratio of 1:1, at least a three-fold difference in relaxation times, and a signal-to-noise ratio (SNR) of at least 20 and preferably 50 or more. A logarithmic rather than linear spacing of TE values is preferred (12-14) as more efficient. The in vivo brain PCr:Cr ratio is near unity (7; 15-18). For the shortest TE of our 3T ^{1}H data for these six subjects, the SNR of the 3.0 ppm methyl resonances of tCr was 26 ± 8 (range 18-41), the SNR of the 3.2 ppm methyl resonances of tCho was 20.5 ± 6.4 (range 12-28), and the SNR of the 2.0 ppm methyl resonance of NAA was 47.5 ± 10.0 (range 36-65). Thus bi-exponential fitting of PCr and Cr components with relaxation times differing threefold or

more should be possible for tCr. Based on this SNR criterion, bi-exponential fitting should be possible for tCho and quite reliable for NAA. It has been speculated that a minimum of 40 TE values and preferably 60 or more are needed for reliable bi-exponential curve fitting (9). When separate T_2s were reported for PCr and Cr at 1.5T (7), 64 TE values (minimum 48 ms), 4 signal averages, and 27 cm^3 voxels were used. When only a single tCr T_2 was observed at 4T (9), 48 TE values (minimum 30 ms), 8 or 16 signal averages, and 8 cm^3 voxels were used. Although SNR values were not reported in these two papers, it is likely that SNR rather than number of TE values used was limiting in the later paper (9) with much smaller voxels. To partially test this, data from Figure 4 of Ke et al. 2002 (7) were re-analyzed, using all, half, and one-third of the data points. This figure shows the signal decay curve as a function of TE for a representative single subject, to demonstrate the bi-exponential decay of tCr and the mono-exponential decay of NAA. The re-analysis results are shown in Table 6 below.

Table 6. T_2 values of total creatine (tCr) and of Cr and PCr methyl peaks at 3.0 ppm from single- and double-exponential fits, respectively, of peak intensity decay curves from 1.5T data of Figure 4 of Ke et al., 2002 (7), with 4 signal averages and 27 cm^3 voxels.

TE Values	1-exp, 2-par	2-exp, 4-par	
	tCr T_2 /ms	tCr (Cr) T_{2A} /ms (f_A)	tCr (PCr) T_{2B} /ms (f_B)
64 (48-678 ms, 10 ms increments)	194.0 ± 4.7	243.3 ± 9.8 (47.2%)	44.1 ± 7.4 (52.8%)
32 (48-668 ms, 20 ms increments)	189.6 ± 7.1	253.0 ± 17.1 (45.7%)	49.4 ± 10.6 (54.3%)
32 (58-678 ms, 20 ms increments)	199.2 ± 6.2	234.3 ± 11.6 (46.7%)	37.1 ± 11.7 (53.3%)
22 (48-678 ms, 30 ms increments)	189.9 ± 8.6	235.8 ± 12.4 (43.2%)	33.8 ± 9.0 (56.8%)
21 (58-658 ms, 30 ms increments)	194.4 ± 7.4	244.0 ± 17.4 (49.8%)	49.3 ± 14.7 (50.2%)
21 (68-668 ms, 30 ms increments)	201.9 ± 8.5	390.2 ± 219.7 (27.9%)	111.8 ± 39.9 (72.1%)
64 (48-678 ms, 10 ms increments)	194.0 ± 4.7	2-exp, 3-par (fixed c/a = 52/48 = 1.08333) [a]	
		239.5 ± 10.3 (52.0%)	48.2 ± 9.0 (48.0%)
64 (48-678 ms, 10 ms increments)	1-exp, 3-par [b]	2-exp, 5-par [c]	
	151.7 ± 5.4	203.1 ± 18.0 (46.3%)	31.3 ± 8.6 (53.7%)

[a] Using a fixed ratio of 52% long-T_2 component and 48% short-T_2 component, after Ke et al. 2002 (7).
[b] Using a 1-exponent, 3-parameter fit ($y = y_0 + ae^{-bx}$), signal-to-noise $a/y_0 = 19$.
[c] Using a 2-exponent, 5-parameter fit ($y = y_0 + ae^{-bx} + ce^{-dx}$), signal-to-noise $(a + c)/y_0 = 78.5$.

The results in Table 6 above indicate that as few as 20 TE values are sufficient for reliable bi-exponential fitting, as long as the TE values span the entire decay curve and the other criteria mentioned above are also satisfied. It also shows the importance of having TE values short enough (in this 1.5T example, less than 60 ms; see row 6 of table) to capture the decay of the faster relaxing component. The next-to-last row of Table 6 shows results of bi-exponential fits when the proportions of the two components are fixed at 52/48, as done for Cr/PCr (7). The last row of Table 6 shows the results from a 1-exponential, 3-parameter fit equation and a 2-exponential, 5-parameter fit equation. Including the finite baseline parameter y_0 rather than forcing decay to zero can improve the fit to the data in cases of inadequate SNR. The results of the last row of Table 6 indicate that although this is the case for the Ke et al. (7) data, a bi-exponential decay is still obtained.

It has been pointed out (12) that non-monoexponential decay of diffusion attenuation curves can arise from two populations within the same cellular compartment with different diffusion coefficients, from two cellular compartments with different environments conferring different diffusion coefficients to species within them, or from restricted diffusion within a compartment. The first two situations are analogous (a) to PCr and Cr within the same tissue type and/or cellular compartment having different T_2 values and (b) to tCr having different T_2 values in different tissue types (e.g., GM and WM) and/or cellular compartments (e.g., cytoplasm and mitochondria) but with the two molecular species having indistinguishable T_2 values within a specific tissue type or cellular compartment. Even in normal variable TE experiments with no diffusion gradients applied, either with a single or with multiple variable gradient strengths or durations, using increments of echo spacing in a single-echo experiment to vary TE, rather than different numbers of echoes with a constant echo spacing, can in theory reduce the ability to detect bi-exponential decay in cases where there are inherent microscopic gradients of the magnetic field within the cell through which the species are diffusing. This is because in the single-echo experiment, with variable echo spacing used to generate different TE values, successively longer echo spacing will include increasingly more signal decay from diffusion through the microscopic gradients, for unrestricted diffusion. It is thus apparent that a series of carefully planned and executed experiments are necessary to distinguish which of the several different circumstances are occurring before unambiguous interpretations of the data are possible, especially in a complex biological system in vivo. This is further complicated in the case of the methyl peaks of tCr because other species, especially GABA and macromolecules, have resonances in the 3.0 ppm region which overlap those of PCr and Cr in the ^{1}H spectrum.

The data above collected at UT Southwestern Medical Center indicated that data analyses needed to be done to test the effect of macromolecular (MM) overlap on the 3.0 ppm tCr methyl resonance, by subtracting the MM contribution, as mentioned by Ke et al. (7), following Behar and colleagues (19, 20). The GABA contribution to the 3.0 ppm resonance region has been calculated to be <5%, negligibly small (7), but it also can be modeled.

After the P.I. moved to Georgia State University, additional multiple-TE data were collected on the 3T Siemens system using an ultra-short echo (minimum TE = 5 ms) STEAM sequence. Spectra were modeled with LCModel, with metabolite spectra (including those for GABA and MM) modeled by theoretical simulations with VESPA. For the three subjects who provided high-quality data with minimal motion artifacts, no hint of bi-exponential transverse relaxation of the 3.0 ppm tCr peak was observed (33; see Appendix 2), adding further evidence to the data collected on the 3T Philips system at UT Southwestern and the literature (9) that suggested this might be difficult or impossible at field strengths of 3T and above.

Task 4. Recruitment and scheduling of Gulf War veterans (**months 5-24**)
4a. Recruit and schedule Gulf War veterans from the national sample of 97 Gulf War veterans tested in 2009-2010 (**months 5-23**).
> ➢ Work on this task was begun (by Dr. Haley), but then halted when it was decided to close the grant, due in part to the issues described for Tasks 2 and 3.
4b. Make financial payments and expense reimbursements (**months 5-24**).
> ➢ This was done for the normal volunteers recruited for the protocol and parameter optimization experiments conducted in the pilot phase.

Task 5. Collection of ^{1}H and ^{31}P MRS data from Gulf War veterans (**months 6-23**)
5a. Year 1 target is collection of data from 31 veterans (**months 6-12**).

> ➢ Work on this task was not done.

5b. Year 2 target is collection of data from an additional 66 veterans (**months 13-23**).

> ➢ Work on this task has not done.

Task 6. Analysis and reporting of ^{1}H and ^{31}P MRS data from Gulf War veterans (**months 6-24**)

6a. Transfer ^{1}H and ^{31}P MRS data to an off-line computer for analysis and archiving (**months 6-24**).

> ➢ Work on this task was not done.

6b. Interpret the data, relate it to prior data, and write reports and papers (**months 13-24**).

> ➢ Work on this task was not done.

Problem Areas

A. ^{31}P Performance of Dual-tuned ^{31}P-^{1}H Head Coil

There was a temporary problem with ^{31}P coil performance when the dual-tuned ^{31}P-^{1}H head coil was first delivered that persisted after it was sent back to the manufacturer for repair and returned to UT Southwestern. Cable connections in the 3T Philips MR system were found to be faulty; when this was fixed, the circumstances improved but intermittent SNR deficiencies still remained. The coil was again sent back to the manufacturer and a faulty connector in the transmit/receive (T/R) switch was discovered and fixed, eliminating the intermittent issues.

B. Ability to Detect Individual Cr and PCr T_2 Components with ^{1}H MRS

A potential problem exists with the planned measurement of bi-exponential relaxation of the tCr peak with ^{1}H MRS to resolve Cr and PCr. This approach was proposed based upon the reported nearly three-fold (117 ms for PCr and 309 ms for Cr, measured at 1.5T with 64 TE values) difference in T_2 values for the overlapping PCr and Cr methyl peaks constituting the tCr methyl resonance (7). However, a more recent experiment by the same laboratory (9) performed at 4T with 48 TE values failed to detect the individual Cr and PCr transverse relaxation times, instead measuring only a single averaged component as is typically reported in the literature. This failure was blamed upon the fewer TE values used compared to the original report that successfully obtained individual Cr and PCr T_2 values. As demonstrated above, it is likely that SNR rather than fewer TE values was limiting in the experiments reported in the later paper (9), which used much smaller voxels.

The inability of Ongur et al. (9) to reproduce the findings of Ke et al. (7) spurred us to investigate the equations governing the transverse relaxation of Cr and PCr under conditions of chemical exchange mediated by creatine phosphokinase (CPK), as occurs *in vivo*, using realistic parameters from the literature to determine the theoretical feasibility of detecting and measuring the two individual Cr and PCr T_2 relaxation components.

There are two questions of interest in the following calculations pertinent to Cr and PCr. The first and most important, from the perspective of our proposed experiments, is whether or not the PCr ↔ Cr exchange rates are fast or slow on the spectroscopic (compared to the separation of the Cr and PCr methyl peaks) and transverse relaxographic (compared to the sum of the transverse relaxation rates of the two exchanging species) time scales. This will dictate whether both individual transverse relaxation components can be measured (slow regime) or whether a single average transverse relation rate is measured (fast regime). The second is whether the exchange

contribution to the transverse relaxation is insignificant or not; if it is not insignificant, then it might be desirable to use a very short echo spacing (τ_{CPMG}) to effectively remove its contribution, or to vary τ_{CPMG} or use a very short and a very long value of τ_{CPMG} to quantify the contribution of the exchange rate to the overall transverse relaxation.

The measured transverse relaxation rate $R_{2,CPMG}$ measured as a function of the Carr-Purcell-Meiboom-Gill (CPMG) echo spacing τ_{CPMG} is given by the Luz-Meiboom (LM) equation (21):

$$R_{2,CPMG} = A + B[1 - (2C/\tau_{CPMG})\tanh(\tau_{CPMG}/2C)]$$

where $A = R_{2M}$ is the observed or measured (average) transverse relaxation rate absent exchange (or in the presence of exchange but when τ_{CPMG} is so short as to approach zero, thus effectively removing the exchange contribution from observation), $B = P_A P_B (\Delta\omega)^2 \tau_{ex}$ is the exchange contribution to the measured transverse relaxation rate, and $C = \tau_{ex}$ is the exchange lifetime. In the expression for B, P_A and P_B are the relative populations of the two exchanging species (here Cr and PCr) and $\Delta\omega$ is the separation (in Hz or s^{-1}) between the Cr and PCr methyl peaks in the ^1H spectrum.

CPK-mediated exchange between Cr and PCr on the spectroscopic time scale. The relative magnitudes of the spectroscopic shutter-speed $\Delta\omega \equiv |\omega_A - \omega_B|$, the frequency separation in Hz of two peaks in an MR spectrum, and of the exchange rate constant τ^{-1}, where $\tau^{-1} \equiv \tau_{PCr}^{-1} + \tau_{Cr}^{-1}$ and τ_{PCr} and τ_{Cr} are the lifetimes of the methyl spins in the two sites, dictate whether two peaks with separately measurable relaxation times are observable or a single merged peak is observed (22). If $\Delta\omega \gg \tau^{-1}$ (the slow-exchange-limit or SXL condition, or the no-exchange-limit or NXL condition), two peaks are seen. In the intermediate exchange regime ($\Delta\omega \approx \tau^{-1}$), two partially overlapping peaks are detected. If $\Delta\omega \ll \tau^{-1}$ (the fast-exchange-limit or FXL condition), one spectroscopic peak (resonance frequency) is seen.

The separation $\Delta\omega$ between the PCr and Cr ^1H methyl peaks is 0.0020 ppm (23), which in frequency units is 0.128 Hz at 1.5T, 0.255 Hz at 3T, 0.340 Hz at 4T, and 0.596 Hz at 7T. By comparison, the forward rate constant k_f(PCr→Cr+ATP) for the CPK-mediated reaction in human brain is 0.3 s^{-1} (24-26) and the reverse rate constant k_r(Cr+ATP→ PCr) is 0.42 ± 0.05 s^{-1} (25). The exchange rate term $1/C = 1/\tau_{ex}$ in the LM equation equals k_f(PCr→Cr+ATP) + k_r(Cr+ATP→ PCr) which sums to 0.72 s^{-1}. Thus the exchange regime is intermediate-fast for low field (1.5T) and intermediate for intermediate field (3T, 4T) and for high field (7T). These calculations indicate that, on the spectroscopic time scale, it would be difficult to resolve the individual PCr and Cr methyl peaks to measure their separate transverse relaxation components unless an ultra-high field magnet (>10T) were used. This is well-know and obvious from experimental spectra.

CPK-mediated exchange between Cr and PCr on the transverse relaxation time scale. Even if two spectral peaks are superimposed and can't be resolved, as is the case for the PCr and Cr methyl peaks at practically available field strengths, separate relaxation times T_1 and/or T_2 can still be observed as a bi-exponential relaxation curve under appropriate circumstances. This has been explained in terms of the <u>relaxographic</u> shutter speed, τ_X^{-1}, defined for the case of PCr and Cr as $|R_{XPCr} - R_{XCr}|$, where $R_X \equiv 1/T_X$, and X = 1 for longitudinal relaxation and 2 for transverse relaxation (12, 27-29). The recovery exponentiality depends on the relative magnitudes of τ_X^{-1} and the exchange rate constant, τ^{-1}, where $\tau^{-1} \equiv \tau_{PCr}^{-1} + \tau_{Cr}^{-1}$ and τ_{PCr} and τ_{Cr} are the lifetimes of the methyl spins in the two sites. If $\tau_X^{-1} \ll \tau^{-1}$ (the FXL condition), the

relaxation will be averaged and the recovery will be mono-exponential. If $\tau_X^{-1} >> \tau^{-1}$ (the SXL condition or the NXL condition), the recovery will be non-mono-exponential and individual relaxation components can be measured. Transverse shutter-speed (τ_2^{-1}) values are typically larger than longitudinal shutter-speed (τ_1^{-1}) values and thus, in general, it is easier to depart the FXL condition into non-mono-exponentiality for T_2 than for T_1.

Multiple single-site ^{31}P saturation magnetization transfer experiments of human occipital lobe performed at 7T yielded CPK exchange rate constants of $k_f = 0.30 \pm 0.04$ s^{-1} = τ_{PCr}^{-1} for PCr + ADP + H$^+$ → Cr + ATP and $k_r = 0.42 \pm 0.05$ s^{-1} = τ_{Cr}^{-1} for Cr + ATP → PCr + ADP + H$^+$ (25). Thus $\tau^{-1} = \tau_{PCr}^{-1} + \tau_{Cr}^{-1} = 0.30$ s^{-1} + 0.42 s^{-1} = 0.72 s^{-1}. From the literature report of bi-exponential tCr T_2 behavior at 1.5T in human brain (7), Cr T_2 = 309 ± 21 ms and PCr T_2 = 117 ± 21 ms; thus the <u>relaxographic</u> shutter speed, τ_2^{-1} = $|R_{2PCr} - R_{2Cr}|$ = $|8.55$ s^{-1} − 3.24 s$^{-1}|$ = 5.3 s^{-1}. Since τ_2^{-1} (= 5.3 s^{-1}) >> τ^{-1} (= 0.72 s^{-1}), this is consistent with the theory predicting SXL and bi-exponential behavior.

Contribution of exchange to the transverse relaxation. At 1.5T in human brain, T_2 has been reported to be 309 ± 21 ms for Cr, 117 ± 21 ms for PCr, and 222 ± 14 ms for the averaged tCr T_2 (7). So the average transverse relaxation rate absent exchange A = R_{2M} = tCr R_2 = (tCr T_2)$^{-1}$ = $(0.222$ s)$^{-1}$ = 4.5045 s^{-1}. For human brain, P_{PCr} = 0.48 and P_{Cr} = 0.52, so $P_A \approx P_B$ (7 and references therein). Since τ_{ex} = C = 1/ k_f(PCr→ATP) = 1/0.3 s^{-1} = 3.3 s, the exchange contribution to the transverse relaxation rate B = $P_A P_B (\Delta\omega)^2 \tau_{ex}$ = (0.48)(0.52)(0.128 s^{-1})^2(3.3 s) = 0.0135 s^{-1}. Because A (= 4.5045 s^{-1}) >> B (= 0.0135 s^{-1}), the exchange contribution to transverse relaxation is negligible. The same holds true at 3T (A (= 7.97 s^{-1}) >> B (= 0.054 s^{-1}), 4T (A (= 6.85 s^{-1} for ACC and 8.06 s^{-1} for POC) >> B (= 0.0952 s^{-1}), and 7T (A (= 11.11 s^{-1}) >> B (= 0.2926 s^{-1}). From UTSW data at 3T for basal ganglia, tCr T_2 = 125.5 ± 7 ms so A = R_{2M} = $(0.1255$ s)$^{-1}$ = 7.97 s^{-1}. For 4T, tCr T_2 = 146 ± 22 ms in ACC and in 124 ± 29 ms in POC, so A = R_{2M} = $(0.146$ s)$^{-1}$ = 6.85 s^{-1} for ACC and for $(0.124$ s)$^{-1}$ = 8.06 s-1 for POC (7). From literature data for basal ganglia at 7T (30), tCr T_2 = 90 ± 11 ms so A = R_{2M} = $(0.090$ s)$^{-1}$ = 11.11 s^{-1}.

The section two paragraphs prior indicates that for human brain in vivo, the CPK reaction exchanging PCr and Cr is likely in the SXL and thus bi-exponential behavior should be observable if other conditions are optimal (10-12), particularly if there is at least a three-fold difference in relaxation times and if 20 > SNR > 50 (or more). There are two alternatives to PCr and Cr bi-exponential T_2 determinations: (1) Chemical exchange saturation transfer (CEST) with ^{1}H imaging may be an alternate way to estimate Cr and PCr (31). However, this is a new method only recently introduced and feasibility tested only for Cr. It would require time to implement and test this technique for both Cr and PCr. (2) ^{31}P magnetization transfer studies of brain CPK and APTase kinetics would complement and augment the ^{31}P MRS studies of brain that were originally proposed for this project. This alternative is attractive because it would make use of the ^{31}P-^{1}H dual-tuned head coil, which is not commonly available at most MR sites. However, the PI moved to Georgia State University, which has a 3T Siemens MR system lacking multinuclear capability, so the ^{31}P experiments were not possible there. Instead, further multi-TE experiments were done as originally proposed, in an effort to measure bi-exponential decay of the tCr methyl signal and determine PCr and Cr T_2 values.

C. Available Time on and Access to UT Southwestern AIRC 3T Philips MR System

When planning for this project began, time was much more available on the UT Southwestern AIRC 3T Philips MR system than when the project commenced. Reserving time was more

difficult and challenging than anticipated when the accelerated two-year grant period was chosen. To help this situation, the ^{1}H protocol was tested and set up on both AIRC 3T Philips systems. Because of the problems with the dual-tuned ^{31}P-^{1}H coil and with getting time on the AIRC 3T systems, the project fell 6 months behind schedule. About 9 months was required for the transfer of the grant to Georgia State University, requiring the performance period to be extended a year, making its duration three rather than two years.

KEY RESEARCH ACCOMPLISHMENTS

➢ Theoretical calculations performed using parameter estimates from the literature indicated that the CPK-catalyzed chemical exchange of phosphate between PCr and Cr at 3T is in the fast exchange limit on the spectroscopic time scale and in the slow exchange limit on the transverse relaxation time scale, and that the exchange process contributes negligibly to the ^{1}H 3.0 ppm tCr methyl peak transverse relaxation.

➢ Experiments to define the best 3T parameters for measuring bi-exponential T_2 relaxation of the methyl peak of tCr in brain at 3.0 ppm indicated that (1) TEs shorter than about 40 ms are needed to adequately quantify the faster relaxing component; (2) as few as 20 TE values are sufficient for reliable bi-exponential fitting, as long as the TE values span the entire decay curve and adequate SNR (20-50) is obtained; (3) including a finite baseline parameter rather than forcing decay to zero may improve the fit to the data in cases of marginal SNR. The first result suggests that STEAM, which can allow substantially shorter TEs than PRESS, may be advantageous despite its lower sensitivity per unit time. To ensure that T2 differences in gray matter and white matter (GM and WM) are not confounding, data from a voxel in WM centrum semiovale were used for the T_2 relaxation time determinations.

➢ Experiments at 3T following these criteria and using an ultra-short TE STEAM sequence capable of a minimum TE of 5 ms failed to show evidence of bi-exponential transverse relaxation in the 3.0 ppm tCr peak in brain white matter.

CONCLUSION

Several research results were obtained (see "Key Research Accomplishments" section above) which indicated that detection of bi-exponential transverse relaxation of the 3.0 ppm tCr methyl peak with ^{1}H MRS would be difficult, if not impossible, at 3T. Coupled with the P.I.'s move to Georgia State University in Atlanta in November of 2013, where the 3T Siemens MR system was not capable of doing ^{31}P MRS, it was decided to prematurely terminate the grant before Gulf War veterans were recruited for study.

PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS

1. Lay Press: None.
2. Peer-reviewed Scientific Journals: None.
3. Invited Articles: None.
4. Abstracts:
   L.C. Krishnamurthy, S. Cheshkov, and R.W. Briggs. On the bi-exponential decay of tCr transverse magnetization in brain in vivo. Abstract 6320 submitted 11/12/14, 23rd meeting of the International Society of Magnetic Resonance in Medicine (ISMRM), Toronto, Ontario, Canada, May 30-June 5, 2015.
5. Presentatations: None.

INVENTIONS, PATENTS, AND LICENSES

None.

REPORTABLE OUTCOMES

➢ No manuscripts have yet resulted from this research. One meeting abstract (see Appendix 2) has been submitted, but word has not been received from the review that determines whether this submitted abstract will be accepted for presentation.

➢ No inventions, patents, or licenses have been applied for or issued.

OTHER ACHIEVEMENTS

➢ No degrees have been obtained that are supported by this award;

➢ No cell lines or tissue or serum repositories have been developed;

➢ No informatics such as databases or animal models have been developed;

➢ The P.I. was recruited and hired to a senior faculty position at Georgia State University and moved to Atlanta in November of 2013 based in part on this award and the experience it provided. In addition, the P.I. was actively recruited for a faculty position at the University of Florida and the VA Medical Center in Gainesville, Florida based in part on experience doing Gulf War Illness research supported by this award.

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APPENDICES

Appendix 1. List of Personnel Receiving Pay from the Research Project

Appendix 2. Krishnamurthy LC, Cheshkov S, Briggs RW. On the bi-exponential decay of tCr transverse magnetization in brain in vivo. Abstract 6320 submitted 11/12/14, 23rd meeting of the International Society of Magnetic Resonance in Medicine (ISMRM), Toronto, Ontario, Canada, May 30-June 5, 2015

Appendix 1

List of Personnel Receiving Pay from the Research Project

Briggs, Richard W. (Principal Investigator, UTSWMC and GSU)

Cheshkov, Sergey (Co-investigator, UTSWMC)

Haley, Robert W. (Co-investigator, UTSWMC)

Krishnamurthy, Lisa C. (Postdoctoral Researcher, GSU)

McColl, Roderick W. (Co-investigator, UTSWMC)

Reeves, Melody (Administrative Assistant, UTSWMC)

Appendix 2

Krishnamurthy LC, Cheshkov S, Briggs RW. On the bi-exponential decay of tCr transverse magnetization in brain in vivo. Abstract 6320 submitted 11/12/14, 23rd meeting of the International Society of Magnetic Resonance in Medicine (ISMRM), Toronto, Ontario, Canada, May 30-June 5, 2015

On the Bi-exponential Decay of tCr Tranverse Magnetization in Brain In Vivo

Lisa C. Krishnamurthy[1,2], Sergey Cheshkov[3], and Richard W. Briggs[1,2]

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Introduction In ^{1}H MRS, even at ultra-high field, it is impossible to spectrally resolve the completely overlapping methyl group resonances of creatine (Cr) and phosphocreatine (PCr). It has been reported that these two resonances can be distinguished by their transverse relaxation rates (1). A biexponential fit of the 3.0 ppm total creatine (tCr = Cr + PCr) decay curve collected at 1.5 T, characterized by 64 TE values between 48 ms and 678 ms, yielded two T_2 values for the 3.0 ppm resonance in both phantom solutions (78 ms for PCr and 153 ms for Cr, for a 3% BSA solution doped with 0.5 mM $MnCl_2$) and brain in vivo (117 ± 21 ms for PCr and 309 ± 21 ms for Cr, with the constraint that PCr/Cr = 48/52). This means that group differences in relative amounts of PCr and Cr, or inter-conversion of PCr and Cr could lead to differences or changes in the tCr peak intensity. Group differences or temporal changes in PCr and/or Cr T_2 values could further complicate matters, creating inaccuracies and ambiguities when the tCr resonance is used as an internal concentration reference or standard. A more recent experiment (2) performed at 4T with 48 TE values between 30 ms and 500 ms failed to detect the individual Cr and PCr transverse relaxation times, instead measuring only a single averaged component as is typically reported in the literature. This failure was attributed to the lesser number of TE values used compared to the original report (1). The aim of this study is to further evaluate the conditions for detecting the possible bi-exponential transverse relaxation of the 3.0 ppm resonance peak region, which predominantly is characterized by tCr, but also contains small contributions from GABA and macromolecules (1).

Methods Data from Fig. 4 of (1) were manually transcribed for re-analysis. Human brain data were obtained for T2 analyses with STEAM localization, two from a Philips 3T (white matter centrum semiovale; 4.0x.1.8x1.8 = 13.0 cm^3; 32 averages; TR = 3 s; 40 log-spaced TEs from 10-600 ms) and three from a Siemens 3T (white matter centrum semiovale; 4.0x.1.7x1.5 = 10.2 cm^3; 64 averages except for 96, 128, and 160 for the last three TEs; TR = 3 s; 18 log-spaced TEs from 5-500 ms). The data were pre-processed in VESPA by removing the residual water with SVD filtering, and applying a 5Hz line-broadening to improve SNR. The pre-processed data was converted to .RAW files, and analyzed with LCModel using a unique basis set at each TE that included Ala, Asp, Cr, PCr, GABA, Glc, Gln, Glu, GPC, PCh, GSH, Ins, Lac, NAA, NAAG, Scyllo, and Tau metabolites, as well as lipid and macromolecule (MM) resonances. Default LCModel fit parameters were used (including unconstrained baseline) over a range of 0.2 - 6.2 ppm.

Results Table 1 shows results of re-analyses of data from Figure 4 of (1) using all, half, and one-third of the data points. The results indicate that as few as 20 TE values are sufficient for reliable bi-exponential fitting, as long as the TE values span the entire decay curve and other criteria (discussed below) are also satisfied. It also shows the importance of having TE values short enough (< 60 ms for 1.5T; row 6) to capture the decay of the faster relaxing component. For the 5 subjects tested at 3T, the data showed no evidence of bi-exponential decay. Figure 1 is a representative dataset for data acquired for this study.

Table 1. T_2 values of total creatine (tCr) and of Cr and PCr methyl peaks at 3.0 ppm from single- and double-exponential fits, respectively.

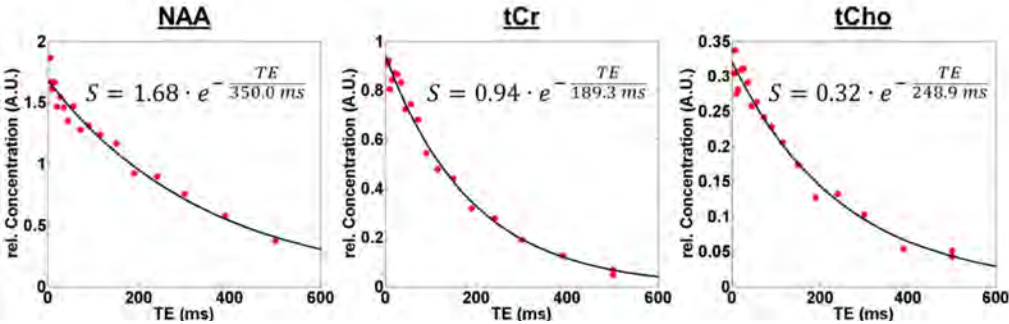| TE Values	1-exp, 2-par	2-exp, 4-par	
	tCr T_2 /ms	tCr (Cr) T_{2A} /ms (f_A)	tCr (PCr) T_{2B} /ms (f_B)
64 (48-678 ms, 10 ms increments)	194.0 ± 4.7	243.3 ± 9.8 (47.2%)	44.1 ± 7.4 (52.8%)
32 (48-668 ms, 20 ms increments)	189.6 ± 7.1	253.0 ± 17.1 (45.7%)	49.4 ± 10.6 (54.3%)
32 (58-678 ms, 20 ms increments)	199.2 ± 6.2	234.3 ± 11.6 (46.7%)	37.1 ± 11.7 (53.3%)
22 (48-678 ms, 30 ms increments)	189.9 ± 8.6	235.8 ± 12.4 (43.2%)	33.8 ± 9.0 (56.8%)
21 (58-658 ms, 30 ms increments)	194.4 ± 7.4	244.0 ± 17.4 (49.8%)	49.3 ± 14.7 (50.2%)
21 (68-668 ms, 30 ms increments)	201.9 ± 8.5	390.2 ± 219.7 (27.9%)	111.8 ± 39.9 (72.1%)

Figure 1 LCModel analysis of 3T data acquired for this study. The relative concentration of NAA (=NAA+NAAG), tCr, and tCho (=GPC+PCh) are plotted with respect to TE (red circles). The mono-exponential fit (black curve) describes the data well (equation in inset). The bi-exponential fit resulted in a similar T2, with half the signal intensity prescribed to each component.

Discussion It has been speculated that a minimum of 40 TE values (preferably 60) are needed for reliable bi-exponential curve fitting (2). However, our reanalysis of the 1.5T data from (1) suggests that the number of TEs may not be as important as sampling the entire decay curve (including measuring signal at short TEs). Although SNR values were not reported in (1) and (2), it is also likely that SNR or B_0-dependent T_2 differences, rather than number of TE values used was limiting in the later paper (2), which acquired much smaller voxels. Furthermore, LCModel spectral fitting may also lessen the contribution of GABA and MM resonances at 3.0 ppm, which could artificially suggest bi-exponential behavior of tCr. Optimal data conditions for successful and reliable fitting of bi-exponential data have been defined (3-5) to be a signal contribution ratio of 1:1, a (minimum) three-fold difference in relaxation times, and a signal-to-noise ratio (SNR) of at least 20 (but preferably 50). A logarithmic rather than linear spacing of TE values is more efficient (5-7). The in vivo brain PCr:Cr ratio is near unity (1; 8-11), so the combination of just-adequate SNR (SNR=16-23 for data from this study) and the possibility of more similar T2s for PCr and Cr at high field may explain the inability to detect the bi-exponential behavior previously reported (1).

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